Supplementary Material

Calprotectin blockade inhibits long-term vascular pathology following peritoneal dialysis-associated bacterial infection

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Supplementary Table 1. Effect of *S. epidermidis* peritonitis on aortic atherosclerosis-associated gene expression at Day 28

Gene Symbol	Description	Fold Change	*p*
Apoa1	Apolipoprotein A-I	21.7	*0.0001*
Apob	Apolipoprotein B	11.4	*0.0008*
Ccn2	Cellular communication network factor 2	2.3	*0.0001*
Eln	Elastin	2.3	*0.0001*
Fga	Fibrinogen alpha chain	26.3	*0.0001*
Fgb	Fibrinogen beta chain	22.3	*0.0001*
Fn1	Fibronectin 1	2.1	*0.0001*
Hbegf	Heparin-binding EGF-like growth factor	2.3	*0.0002*
Il4	Interleukin 4	2.2	*0.0062*
Itga5	Integrin alpha 5 (fibronectin receptor alpha)	2.1	*0.0001*
Lif	Leukemia inhibitory factor	2.1	*0.0080*
Serpine1	Serine (or cysteine) peptidase inhibitor, clade E, member 1	2.8	*0.0002*
Tgfb2	Transforming growth factor, beta 2	2.5	*0.0011*
Tnc	Tenascin C	2.0	*0.0001*
Vwf	Von Willebrand factor homolog	2.6	*0.0001*
Apoe	Apolipoprotein E	-2.2	*0.0001*
Ccr2	Chemokine (C-C motif) receptor 2	-2.0	*0.0001*
Il5	Interleukin 5	-6.1	*0.0047*
Ppara	Peroxisome proliferator activated receptor alpha	-2.1	*0.0001*

Only statistically significant ($p<0.05$) ≤-2 (in green) or ≥ 2 (in red) fold changes compared to PBS control group are shown.

Supplementary Table 2. Effect of *S. epidermidis* peritonitis combined to PDF exposure, or each separately, on aortic atherosclerosis-associated gene expression at Day 28

Gene Symbol	Description	PDF + *S. epi*		*S. epi*		PDF	
		Fold Change*	*p*	Fold Change*	*p*	Fold Change*	*p*
Abca1	ATP-binding cassette, sub-family A (ABC1), member 1	2.1	0.0001	1.8	0.0029	3.2	0.0001
Apoa1	Apolipoprotein A-I	4.6	0.0001	-4.5	0.0112	-1.1	0.5916
Apoe	Apolipoprotein E	2.1	0.0002	2.6	0.0001	-1.4	0.0027
Bcl2a1a	B-cell leukemia/lymphoma 2 related protein A1a	2.4	0.0001	1.8	0.0010	1.2	0.1023
Bid	BH3 interacting domain death agonist	8.2	0.0065	3.1	0.0001	7.7	0.0005
Birc3	Baculoviral IAP repeat-containing 3	4.0	0.0002	1.1	0.4106	2.4	0.0003
Ccl2	Chemokine (C-C motif) ligand 2	5.9	0.0235	5.0	0.0049	4.0	0.0005
Ccl5	Chemokine (C-C motif) ligand 5	10.7	0.0001	-1.1	0.8549	3.3	0.0030
Ccr2	Chemokine (C-C motif) receptor 2	2.3	0.0053	-1.4	0.1991	-1.8	0.3771
Cdh5	Cadherin 5	2.7	0.0000	1.7	0.0055	2.2	0.0033
Cflar	CASP8 and FADD-like apoptosis regulator	2.1	0.0002	1.0	0.4910	1.7	0.0035
Eng	Endoglin	2.1	0.0004	1.3	0.0034	2.7	0.0001
Fas	Fas (TNF receptor superfamily member 6)	2.2	0.0006	1.3	0.1237	2.1	0.0003
Fga	Fibrinogen alpha chain	4.8	0.0025	-3.5	0.1401	1.7	0.3013
Fgb	Fibrinogen beta chain	6.1	0.0001	-3.6	0.0302	1.1	0.7703
Icam1	Intercellular adhesion molecule 1	2.4	0.0002	2.0	0.0002	1.6	0.0089
Ifng	Interferon gamma	3.5	0.0098	-1.2	0.0325	-1.4	0.0060
Il1a	Interleukin 1 alpha	4.5	0.0114	1.9	0.1577	5.0	0.0011
Il1b	Interleukin 1 beta	9.2	0.0359	3.6	0.1267	1.7	0.2044
Itga2	Integrin alpha 2	3.0	0.0089	1.9	0.1135	2.6	0.0033
Itgax	Integrin alpha X	5.4	0.0333	1.8	0.2391	2.4	0.0090
Itgb2	Integrin beta 2	4.2	0.0001	4.6	0.0003	2.8	0.0012
Kdr	Kinase insert domain protein receptor	2.8	0.0001	1.3	0.0189	1.3	0.0566
Lpl	Lipoprotein lipase	2.3	0.0001	2.6	0.0001	2.7	0.0001
Mmp1a	Matrix metallopeptidase 1a (interstitial collagenase)	2.3	0.0002	3.3	0.0001	2.5	0.0001
Msr1	Macrophage scavenger receptor 1	3.8	0.0077	6.9	0.0001	3.4	0.0031
Nfkb1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	2.1	0.0002	1.2	0.0232	3.1	0.0001
Nr1h3	Nuclear receptor subfamily 1, group H, member 3	3.4	0.0001	2.3	0.0040	5.1	0.0001
Pdgfb	Platelet derived growth factor, B polypeptide	3.7	0.0001	1.6	0.0095	2.5	0.0009
Plin2	Perilipin 2	2.2	0.0001	2.3	0.0001	2.4	0.0001
Ppara	Peroxisome proliferator activated receptor α	2.6	0.0000	1.2	0.0121	2.9	0.0000
Pparg	Peroxisome proliferator activated receptor γ	5.3	0.0030	1.6	0.0175	5.3	0.0001
Rxra	Retinoid X receptor alpha	2.3	0.0001	1.4	0.0359	4.0	0.0000
Sele	Selectin, endothelial cell	2.9	0.0014	1.3	0.2716	1.1	0.6213
Selp	Selectin, platelet	2.1	0.0190	1.9	0.0873	1.5	0.1627
Selplg	Selectin, platelet (p-selectin) ligand	9.5	0.0002	2.9	0.0725	9.1	0.0008
Sod1	Superoxide dismutase 1, soluble	2.5	0.0001	2.0	0.0003	2.1	0.0001
Tnf	Tumor necrosis factor	9.2	0.0002	1.8	0.1647	-1.4	0.0060
Tnfaip3	Tumor necrosis factor, alpha-induced protein 3	8.0	0.0001	3.9	0.0019	4.8	0.0162
Vegfa	Vascular endothelial growth factor A	7.3	0.0001	1.5	0.0019	5.0	0.0001
Ccn2	Cellular communication network factor 2	-2.7	0.0010	-2.0	0.0024	-1.6	0.0167
Fn1	Fibronectin 1	-2.9	0.0002	1.3	0.0024	-1.5	0.0007

Only genes with statistically significant (p<0.05) ≤-2 (in green) or ≥ 2 (in red) fold changes for the combination of peritonitis + PDF exposure are shown.
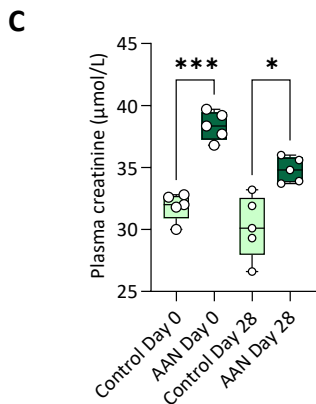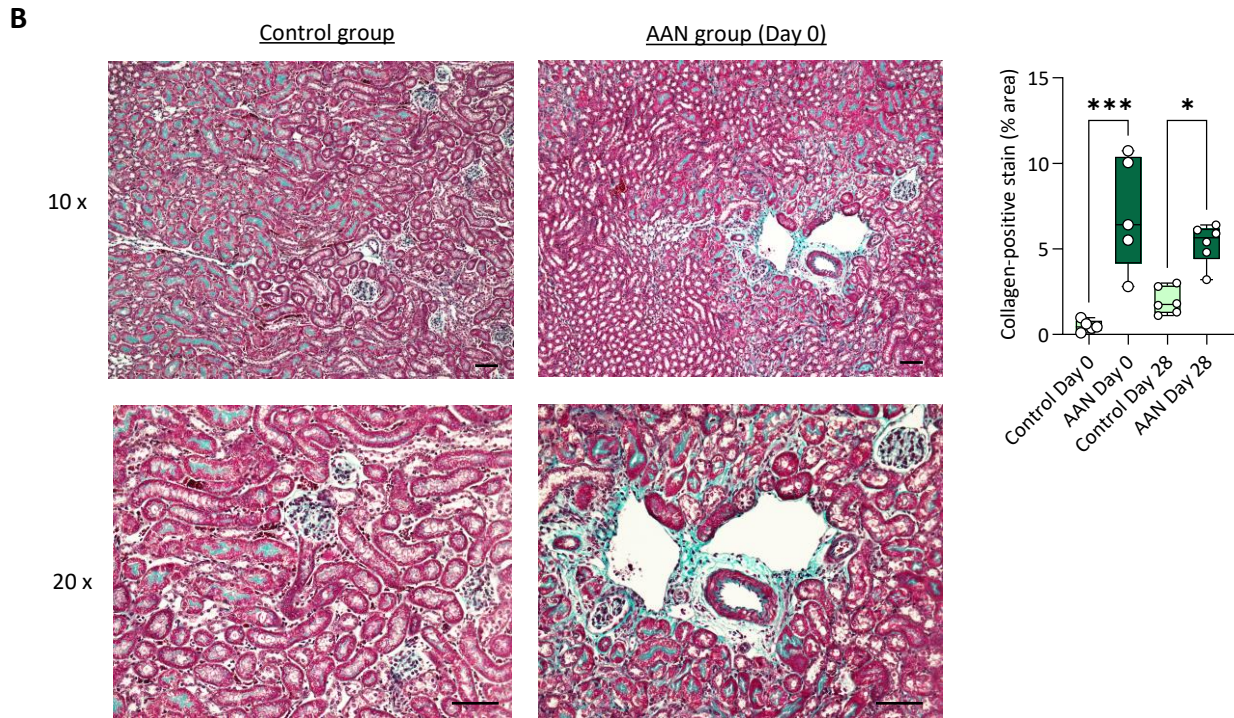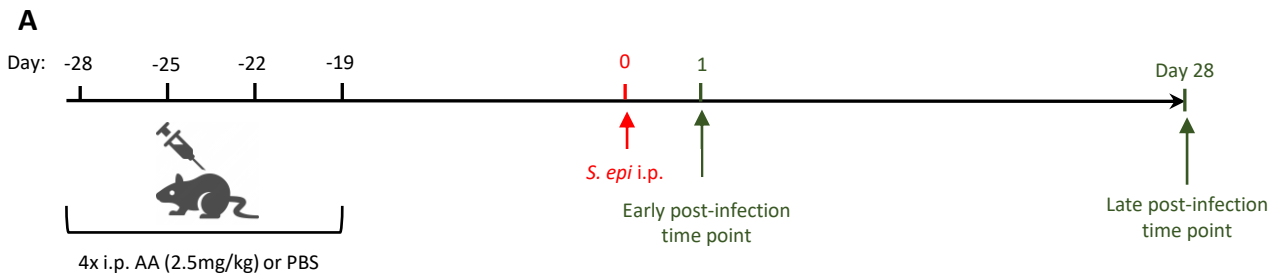**Compared to PBS control group.*

Supplementary Table 3. Effect of Paquinimod administration on *S. epidermidis* peritonitis-induced changes on atherosclerosis-associated gene expression in aortas at Day28

| Gene Symbol	Description	*S. epi*		*S. epi* + Paquinimod	
		Fold Change*	*p*	Fold Change*	*p*
Apoa1	Apolipoprotein A-I	21.7	*0.0001*	2.4	*0.0001*
Apob	Apolipoprotein B	11.4	*0.0008*	1.6	*0.0635*
Ccn2	Cellular communication network factor 2	2.3	*0.0001*	1.2	*0.0005*
Eln	Elastin	2.3	*0.0001*	1.6	*0.0002*
Fga	Fibrinogen alpha chain	26.3	*0.0001*	2.2	*0.0006*
Fgb	Fibrinogen beta chain	22.3	*0.0001*	1.3	*0.2417*
Fn1	Fibronectin 1	2.1	*0.0001*	1.6	*0.0001*
Hbegf	Heparin-binding EGF-like growth factor	2.3	*0.0002*	-1.3	*0.9125*
Il4	Interleukin 4	2.2	*0.0062*	1.6	*0.2511*
Itga5	Integrin alpha 5 (fibronectin receptor alpha)	2.1	*0.0001*	1.5	*0.0003*
Lif	Leukemia inhibitory factor	2.1	*0.0080*	-1.8	*0.0130*
Serpine1	Serine (or cysteine) peptidase inhibitor, clade E, 1	2.8	*0.0002*	1.1	*0.0816*
Tgfb2	Transforming growth factor, beta 2	2.5	*0.0011*	1.1	*0.0118*
Tnc	Tenascin C	2.0	*0.0001*	1.2	*0.0184*
Vwf	Von Willebrand factor homolog	2.6	*0.0001*	-1.4	*0.0013*
Apoe	Apolipoprotein E	-2.2	*0.0001*	-1.2	*0.0022*
Ccr2	Chemokine (C-C motif) receptor 2	-2.0	*0.0001*	-1.1	*0.3971*
Il5	Interleukin 5	-6.1	*0.0047*	-3.3	*0.0147*
Ppara	Peroxisome proliferator activated receptor alpha	-2.1	*0.0001*	1.0	*0.3172*

Only genes with statistically significant (p<0.05) ≤ -2 (in green) or ≥ 2 (in red) fold changes following S. epidermidis administration are shown.
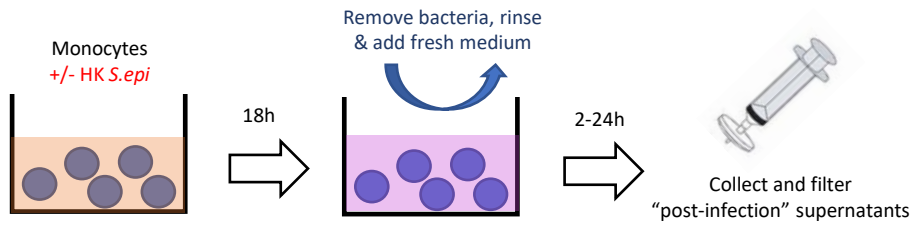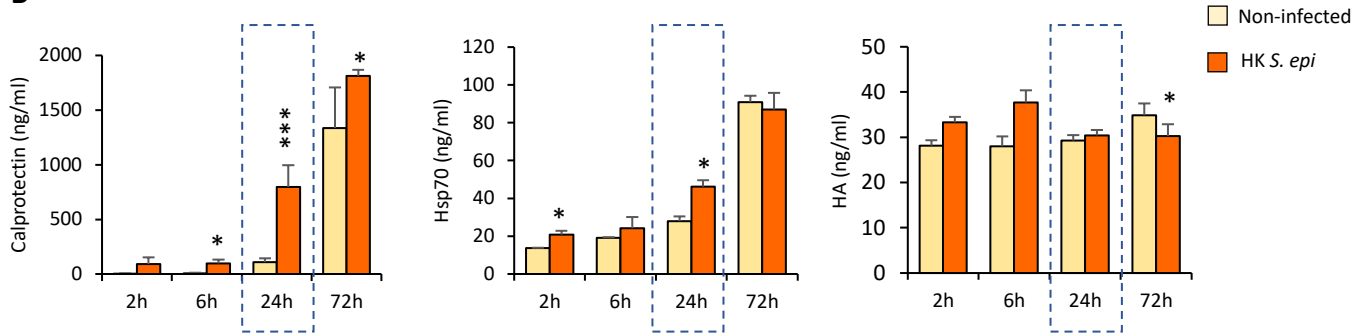
* Compared to PBS control group.

A

Day: -28   -25   -22   -19   0   1   Day 28

4x i.p. AA (2.5mg/kg) or PBS

S. epi i.p.

Early post-infection time point

Late post-infection time point

B

Control group

AAN group (Day 0)

10 x

20 x

C

Supplementary Figure 1. Kidney damage and loss of function following repeated AA injections in mice
A-C C57BL/6J mice (n=5 /group) were injected intraperitoneally with AA (2.5mg/kg) or PBS on days -28, -25, -22 and -19 to induce chronic nephropathy. Day 0 is the day of *S. epidermidis* administration. AAN was verified at Day 0 and Day 28 by the development of kidney fibrosis (**B**) and a significant elevation in plasma creatinine (**C**). **B**. Representative images of Masson trichrome stain of kidneys from a healthy mouse (left panels) and a mouse with chronic AAN (right) . Cytoplasm is stained red, nuclei are in dark brown, and collagen is stained blue, identifying renal fibrosis. Scale bars: 100 μm. Graph shows the percentage of collagen positive stain for each group (3 non-overlapping fields of view scored for each of 5 animals/group) **C**. Creatinine measurements in plasma. *, $p<0.05$; ***, $p<0.005$, ordinary one-way ANOVA (normal distribution) or Kruskal-Wallis test (non-normal distribution).
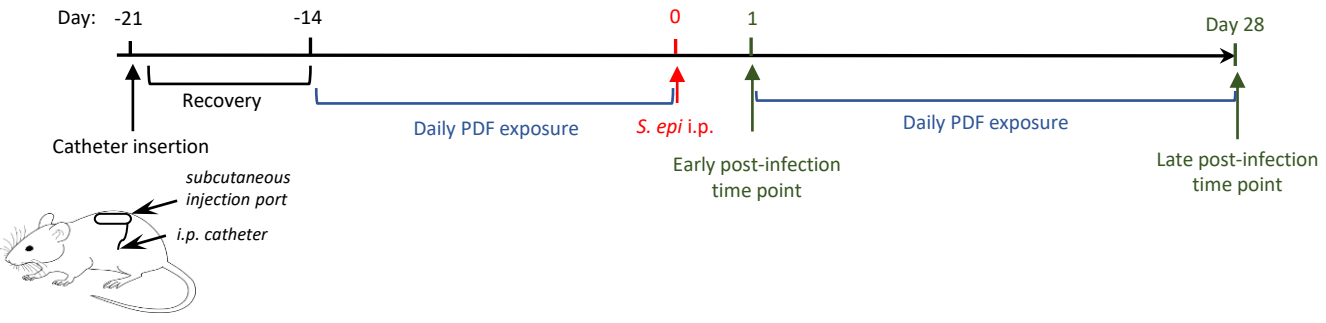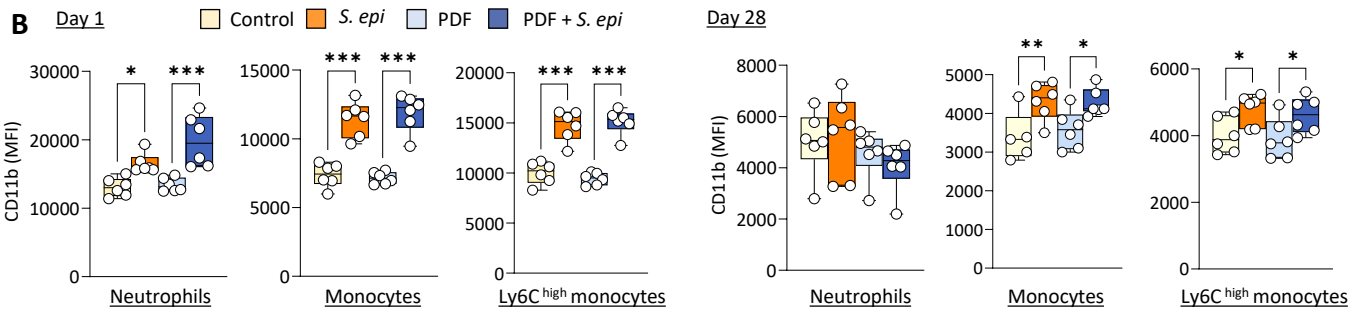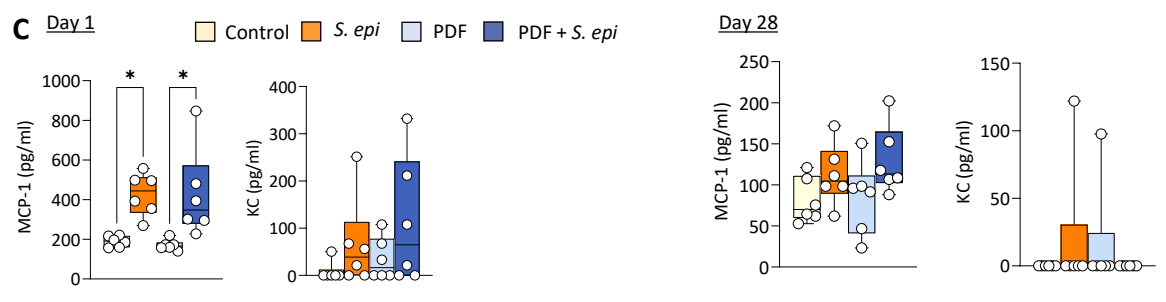
Supplementary Figure 2. Routine PDF exposure in mice does not affect *S. epidermidis*-induced changes in blood innate leukocyte CD11b expression and cytokine levels.

A-C C57BL/6 mice (n=6/ group) were fitted with a peritoneal catheter, given a 7-day recovery period and instilled once daily with 2ml PBS or PDF for 14 day. Mice were then i.p. injected with *S. epidermidis* or PBS (Day 0) and culled at Day 1 or further exposed daily to PBS or PDF, prior to culling at Day 28 (**A**). Blood was collected and expression of CD11b on innate immune leukocytes was determined by flow cytometry (**B**) and plasma levels of cytokines were determined by ELISA (**C**). *, $p<0.05$; **, $p<0.01$; ***, $p<0.005$, ordinary one-way ANOVA (normal distribution) or Kruskal-Wallis test (non-normal distribution).

A

Monocytes
+/- HK *S.epi*

18h

Remove bacteria, rinse
& add fresh medium

2-24h

Collect and filter
"post-infection" supernatants

B

☐ Non-infected
☐ HK *S. epi*

Supplementary Figure 3. Preparation of Calprotectin-containing post-infection supernatants
Mono-Mac 6 monocytes were stimulated (18h) with heat-killed *S. epidermidis* (HK *S. epi*, $5x10^8$cfu/ml) or left untreated. Supernatants were removed, cells were washed and further cultured in complete medium for the indicated durations before post-infection supernatant collection and filtration (0.22μm) (**A**). Calprotectin, Hsp70 and HA levels were determined by ELISA (**B**). 24h post-infection supernatants were selected for use in functional experiments, as the increase in Calprotectin and Hsp70 compared to non-infected supernatants was highest at this time point. *, $p<0.05$; ***, $p<0.005$; HK *S. epi* vs Non-infected, unpaired Student t-test.